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Patents ADP number *(if you know it)* 07157811001

If the applicant is a corporate body, give the country/state of its incorporation

ITALY

4. Title of the invention

COMPOSITION WITH FIVE MENINGOCOCCAL ANTIGENS

5. Name of your agent *(if you have one)*

Carpmaels & Ransford

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

43 Bloomsbury Square
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Patents ADP number *(if you know it)*

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Carpmaels & Ransford **11th October 2002**

12. Name and daytime telephone number of person to contact in the United Kingdom **CAMERON MARSHALL** **020-7242 8692**

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COMPOSITION WITH FIVE MENINGOCOCCAL ANTIGENS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens from *Neisseria meningitidis* and their use in immunisation.

BACKGROUND ART

N.meningitidis is a non-motile, Gram-negative human pathogen that colonises the pharynx and causes meningitis (and, occasionally, septicaemia in the absence of meningitis). It causes both endemic and epidemic disease. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis in the USA.

Based on the organism's capsular polysaccharide, various serogroups of *N.meningitidis* have been identified. Serogroup A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the USA and developed countries.

Genome sequences for *Neisseria meningitidis* (meningococcus) serogroups A [1] and B [2, 3] have been reported. The serogroup B sequence has been studied to identify vaccine antigens [*e.g.* refs. 4 to 9] and candidate antigens have been manipulated to improve heterologous expression [refs. 10 to 12].

Vaccines against pathogens such as hepatitis B virus, diphtheria and tetanus typically contain a single protein antigen (*e.g.* the HBV surface antigen, or a tetanus toxoid). In contrast, acellular whooping cough vaccines typically contain at least three *B.pertussis* proteins, and the Prevenar pneumococcal vaccine contains seven separate conjugated saccharide antigens. Other vaccines such as cellular pertussis vaccines, the measles vaccine, the inactivated polio vaccine (IPV) and meningococcal OMV vaccines are by their very nature complex mixtures of a large number of antigens.

Whether protection against can be elicited by a single antigen, a small number of defined antigens, or a complex mixture of undefined antigens, therefore depends on the pathogen in question.

It is an object of the invention to provide further and improved compositions for providing immunity against meningococcal disease and/or infection. The compositions are based on a combination of at least five meningococcal antigens.

DISCLOSURE OF THE INVENTION

A mixture of five defined protein antigens from serogroup B meningococcus has been found to elicit a good protective immune response. The invention therefore provides a composition comprising the

following five meningococcal protein antigens: (1) a 'NadA' protein; (2) a '741' protein; (3) a '936' protein; (4) a '953' protein; and (5) a '287' protein. These antigens are referred to herein as the 'five basic antigens'.

NadA protein

'NadA' (Neisserial adhesin A) from serogroup B of *N.meningitidis* is disclosed as protein '961' in reference 6 (SEQ IDs 2943 & 2944) and as 'NMB1994' in reference 2 (see also GenBank accession numbers: 11352904 & 7227256). A detailed description of the protein can be found in reference 13. There is no corresponding protein in serogroup A [1, 13].

When used according to the present invention, NadA may take various forms. Preferred forms of NadA are truncation or deletion variants, such as those disclosed in references 10 to 12. In particular, NadA without its C-terminal membrane anchor is preferred (*e.g.* deletion of residues 351-405 for strain 2996 [SEQ ID 1]), which is sometimes distinguished herein by the use of a 'C' superscript *e.g.* $NadA^{(C)}$. Expression of SEQ ID 1 in *E.coli* results in secretion of the NadA protein into the culture supernatant with concomitant removal of its 23mer leader peptide (*e.g.* to leave a 327mer for strain 2996 [SEQ ID 2]). Polypeptides without their leader peptides are sometimes distinguished herein by the use of a 'NL' superscript *e.g.* $NadA^{(NL)}$ or $NadA^{(C)(NL)}$.

Preferred NadA sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 2. This includes NadA variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of NadA are shown in Figure 9 of reference 14.

Other preferred NadA sequences comprise at least *n* consecutive amino acids from SEQ ID 1, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from NadA. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID 1 (*e.g.* $NadA^{(C)}$, $NadA^{(NL)}$, $NadA^{(C)(NL)}$). A preferred fragment of SEQ ID 1 is SEQ ID 2.

Secreted NadA can conveniently be prepared in highly pure form from culture supernatant by a process comprising the steps of: concentration and diafiltration against a buffer by ultrafiltration; anionic column chromatography; hydrophobic column chromatography; hydroxylapatite ceramic column chromatography; diafiltration against a buffer; and filter sterilisation. Further details of the process are given in the examples.

741 protein

'741' protein from serogroup B is disclosed in reference 6 (SEQ IDs 2535 & 2536) and as 'NMB1870' in reference 2 (see also GenBank accession number GI:7227128). The corresponding protein in serogroup A [1] has GenBank accession number 7379322.

When used according to the present invention, 741 protein may take various forms. Preferred forms of 741 are truncation or deletion variants, such as those disclosed in references 10 to 12. In particular, the N-terminus of 741 may be deleted up to and including its poly-glycine sequence (*i.e.* deletion of residues 1 to 72 for strain MC58 [SEQ ID 3]), which is sometimes distinguished herein by the use of a 'ΔG' prefix. This deletion can enhance expression.

Preferred 741 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 3. This includes 741 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 741 can be found in SEQ IDs 1 to 22 of reference 12.

Other preferred 741 sequences comprise at least *n* consecutive amino acids from SEQ ID 3, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 741. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID 3.

936 protein

'936' protein from serogroup B is disclosed in reference 6 (SEQ IDs 2883 & 2884) and as 'NMB2091' in reference 2 (see also GenBank accession number GI:7227353). The corresponding gene in serogroup A [1] has GenBank accession number 7379093.

When used according to the present invention, 936 protein may take various forms. Preferred forms of 936 are truncation or deletion variants, such as those disclosed in references 10 to 12. In particular, the N-terminus leader peptide of 936 may be deleted (*i.e.* deletion of residues 1 to 23 for strain MC58 [SEQ ID 4]) to give $936^{(NL)}$.

Preferred 936 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 4. This includes variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants *etc*).

Other preferred 936 sequences comprise at least *n* consecutive amino acids from SEQ ID 4, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 936. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID 4.

953 protein

'953' protein from serogroup B is disclosed in reference 6 (SEQ IDs 2917 & 2918) and as 'NMB1030' in reference 2 (see also GenBank accession number GI:7226269). The corresponding protein in serogroup A [1] has GenBank accession number 7380108.

When used according to the present invention, 953 protein may take various forms. Preferred forms of 953 are truncation or deletion variants, such as those disclosed in references 10 to 12. In particular, the N-terminus leader peptide of 953 may be deleted (*i.e.* deletion of residues 1 to 19 for strain MC58 [SEQ ID 5]) to give $953^{(NL)}$.

Preferred 953 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 5. This includes 953 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 953 can be seen in Figure 19 of reference 8.

Other preferred 953 sequences comprise at least *n* consecutive amino acids from SEQ ID 5, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 953. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID 5.

287 protein

'287' protein from serogroup B is disclosed in reference 6 (SEQ IDs 3103 & 3104), as 'NMB2132' in reference 2, and as 'GNA2132' in reference 9 (see also GenBank accession number GI:7227388). The corresponding protein in serogroup A [1] has GenBank accession number 7379057.

When used according to the present invention, 287 protein may take various forms. Preferred forms of 287 are truncation or deletion variants, such as those disclosed in references 10 to 12. In particular, the N-terminus of 287 may be deleted up to and including its poly-glycine sequence (*i.e.* deletion of residues 1 to 24 for strain MC58 [SEQ ID 6]), which is sometimes distinguished herein by the use of a 'ΔG' prefix. This deletion can enhance expression.

Preferred 287 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID 6. This includes 287 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 287 can be seen in Figures 5 and 15 of reference 8, and in example 13 and figure 21 of reference 6 (SEQ IDs 3179 to 3184).

Other preferred 287 sequences comprise at least *n* consecutive amino acids from SEQ ID 6, wherein *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 287. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or the N-terminus of SEQ ID 6.

Fusion proteins

The five antigens may be present in the composition as five separate proteins, but it is preferred that at least two of the antigens are expressed as a single polypeptide chain (a 'hybrid' protein [refs. 10 to 12]) *e.g.* such that the five antigens form fewer than five polypeptides. Hybrid proteins offer two

principal advantages: first, a protein that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two separately-useful proteins.

A hybrid protein included in a composition of the invention may comprise two or more (*i.e.* 2, 3, 4 or 5) of the five basic antigens. Hybrids consisting of two of the five basic antigens are preferred.

Within the combination of five basic antigens, an antigen may be present in more than one hybrid protein and/or as a non-hybrid protein. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Two-antigen hybrids for use in the invention comprise: NadA & 741; NadA & 936; NadA & 953; NadA & 287; 741 & 936; 741 & 953; 741 & 287; 936 & 953; 936 & 287; 953 & 287. Preferred two-antigen hybrids comprise: 741 & 936; 953 & 287.

Hybrid proteins can be represented by the formula **NH_2-A-[-X-L-]$_n$-B-COOH**, wherein: X is an amino acid sequence of one of the five basic antigens; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4 or 5.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of [-X-L-], linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID 9), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker. If X_{n+1} is a ΔG protein and L_n is a glycine linker, this may be equivalent to X_{n+1} not being a ΔG protein and L_n being absent.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18,

17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, n is 2. Two preferred proteins of this type are: X_1 is a 936 and X_2 is a 741; X_1 is a 287 and X_2 is a 953.

Two particularly preferred hybrid proteins of the invention are as follows:

n	A	X_1	L_1	X_2	L_2	B	[SEQ ID]
2	MA	ΔG287	GSGGGG	$953^{(NL)}$	—	—	7
2	M	$936^{(NL)}$	GSGGGG	ΔG741	—	—	8

These two proteins may be used in combination with NadA (particularly with SEQ ID 2).

936-ΔG741 hybrid can conveniently be prepared in highly pure form from expression in *E.coli* by a process comprising the steps of: homogenisation; centrifugation; cationic column chromatography; anionic column chromatography; hybrophobic column chromatography; diafiltration against a buffer; and filter sterilisation. Further details of the process are given in the examples.

Sequences

The invention provides a polypeptide having an amino acid sequence selected from the group consisting of SEQ IDs 1 to 8. It also provides polypeptides having an amino acid sequence with sequence identity to an amino acid sequence selected from the group consisting of SEQ IDs 1 to 8. As described above, the degree of 'sequence identity' is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more).

The invention also provides nucleic acid encoding such polypeptides. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*eg.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (*eg.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*eg.* native, fusions, non-glycosylated, lipidated *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other *N.meningitidis* or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other *N.meningitidis* or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA) *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*eg.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Serogroups and strains

Preferred proteins of the invention comprise an amino acid sequence found in *N.meningitidis* serogroup B. Within serogroup B, preferred strains are 2996, MC58, 95N477, and 394/98. Strain 95N477 is sometimes referred to herein as 'ET37', this being its electrophoretic type. Strain 394/98 is sometimes referred to herein as 'nz', as it is a New Zealand strain.

Protein 287 is preferably from strain 2996 or, more preferably, from strain 394/98.

Protein 741 is preferably from serogroup B strains MC58, 2996, 394/98, or 95N477, or from serogroup C strain 90/18311. Strain MC58 is more preferred.

Proteins 936, 953 and 961 are preferably from strain 2996.

Strains may be indicated as a subscript *e.g.* 741_{MC58} is protein 741 from strain MC58. Unless otherwise stated, proteins mentioned herein (*e.g.* with no subscript) are from *N.meningitidis* strain

2996, which can be taken as a 'reference' strain. It will be appreciated, however, that the invention is not in general limited by strain. As mentioned above, general references to a protein (*e.g.* '287', '919' *etc.*) may be taken to include that protein from any strain. This will typically have sequence identity to 2996 of 90% or more (*eg.* 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

Where hybrid proteins are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Heterologous host

Whilst expression of the proteins of the invention may take place in *Neisseria*, the present invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeast *etc.*

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of a composition of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant); where the vaccine is for prophylactic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Neisseria* (*e.g.* meningitis, septicaemia, gonorrhoea *etc.*). The prevention and/or treatment of bacterial or meningococcal meningitis is preferred.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference 15.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other of the above-mentioned components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Dosage treatment may be a single dose schedule or a multiple dose schedule (*e.g.* including booster doses). The vaccine may be administered in conjunction with other immunoregulatory agents.

The vaccine may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Preferred further adjuvants include, but are not limited to: (A) aluminium compounds (*e.g.* aluminium hydroxides, phosphates, aluminium, oxyhydroxide, orthophosphate, sulphate *etc.* [*e.g.* see chapters 8 & 9 of ref. 16]), or mixtures of different aluminium compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous *etc.*), and with adsorption being preferred; (B) MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer) [see Chapter 10 of 16; see also ref. 17]; (C) liposomes [see Chapters 13 and 14 of ref. 16]; (D) ISCOMs [see Chapter 23 of ref. 16], which may be devoid of additional detergent [18]; (E) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion [see Chapter 12 of ref. 16]; (F) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and

one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (G) saponin adjuvants, such as QuilA or QS21 [see Chapter 22 of ref. 16], also known as Stimulon™ [19]; (H) chitosan [*e.g.* 20]; (I) complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA); (J) cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, tumor necrosis factor, *etc.* [see Chapters 27 & 28 of ref. 16]; (K) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) [*e.g.* chapter 21 of ref. 16]; (L) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [21]; (M) a polyoxyethylene ether or a polyoxyethylene ester [22]; (N) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol [23] or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol [24]; (N) a particle of metal salt [25]; (O) a saponin and an oil-in-water emulsion [26]; (P) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [27]; (Q) *E.coli* heat-labile enterotoxin ("LT"), or detoxified mutants thereof, such as the K63 or R72 mutants [*e.g.* Chapter 5 of ref. 28]; (R) cholera toxin ("CT"), or detoxified mutants thereof [*e.g.* Chapter 5 of ref. 28]; (S) double-stranded RNA; (T) microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone *etc.*), with poly(lactide-co-glycolide) being preferred; and (U) oligonucleotides comprising CpG motifs *i.e.* containing at least one CG dinucleotide, with 5-methylcytosine optionally being used in place of cytosine; or (V) other substances that act as immunostimulating agents to enhance the effectiveness of the composition [*e.g.* see Chapter 7 of ref. 16]. Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant toxins are preferred mucosal adjuvants.

Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

Further antigens

The composition contains five basic meningococcal protein antigens. It may also include further antigens, although it may contain no meningococcal protein antigens other than the five basic antigens. Further antigens for inclusion may be, for example:

- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 29 from serogroup C [see also ref. 30] or the oligosaccharides of ref. 31.
- antigens from *Helicobacter pylori* such as CagA [32 to 35], VacA [36, 37], NAP [38, 39, 40], HopX [*e.g.* 41], HopY [*e.g.* 41] and/or urease.
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* 42, 43, 44].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 45, 46].

- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 46, 47].
- an antigen from hepatitis C virus [*e.g.* 48].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 49] *e.g.* the CRM_{197} mutant [*e.g.* 50].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 70].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 51 & 52].
- a saccharide antigen from *Haemophilus influenzae* B [*e.g.* 30].
- polio antigen(s) [*e.g.* 53, 54] such as OPV or, preferably, IPV.
- an antigen from *N.gonorrhoeae* [*e.g.* 55, 56, 57, 58].
- an antigen from *Chlamydia pneumoniae* [*e.g.* refs. 59 to 65].
- an antigen from *Chlamydia trachomatis* [*e.g.* 66].
- an antigen from *Porphyromonas gingivalis* [*e.g.* 67].
- rabies antigen(s) [*e.g.* 68] such as lyophilised inactivated virus [*e.g.* 69, RabAvert™].
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 70].
- influenza antigen(s) [*e.g.* chapter 19 of ref. 70], such as the haemagglutinin and/or neuraminidase surface proteins.
- antigen(s) from a paramyxovirus such as respiratory syncytial virus (RSV [71, 72]) and/or parainfluenza virus (PIV3 [73]).
- an antigen from *Moraxella catarrhalis* [*e.g.* 74].
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 75, 76, 77].
- an antigen from *Staphylococcus aureus* [*e.g.* 78].
- an antigen from *Bacillus anthracis* [*e.g.* 79, 80, 81].
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 82, 83, 84, 85 *etc.*
- an antigen from a virus in the flaviviridae family (genus flavivirus), such as from yellow fever virus, Japanese encephalitis virus, four serotypes of Dengue viruses, tick-borne encephalitis virus, West Nile virus.
- a pestivirus antigen, such as from classical porcine fever virus, bovine viral diarrhoea virus, and/or border disease virus.
- a parvovirus antigen *e.g.* from parvovirus B19.
- a prion protein (*e.g.* the CJD prion protein)
- an amyloid protein, such as a beta peptide [86]
- a cancer antigen, such as those listed in Table 1 of ref. 87 or in tables 3 & 4 of ref. 88.

The composition may comprise one or more of these further antigens.

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity [*e.g.* refs. 89 to 98]. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred [99]. Other carrier polypeptides include the *N.meningitidis* outer membrane protein [100], synthetic peptides [101, 102], heat shock proteins [103, 104], pertussis proteins [105, 106], protein D from *H.influenzae* [107], cytokines [108], lymphokines [108], hormones [108], growth factors [108], toxin A or B from *C.difficile* [109], iron-uptake proteins [110] *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it is preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Saccharides from different serogroups of *N.meningitidis* may be conjugated to the same or different carrier proteins. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [52]).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used [*e.g.* refs. 111 to 119]. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 120. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a

gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in reference 121.

MODES FOR CARRYING OUT THE INVENTION

ΔG287-953 hybrid protein

DNA encoding protein 287 from meningococcal serogroup B strain 394/98 and protein 953 from meningococcal serogroup B strain 2996 were digested and ligated, together with a short linker sequence, to give a plasmid encoding amino acid sequence SEQ ID 7. The plasmid was transfected into *E.coli* and bacteria were grown to express the protein.

After adequate growth, bacteria were harvested and the protein was purified. From culture, bacteria were centrifuged and the pellet was homogenized in the presence of 50 mM acetate buffer (pH 5) with a pellet:buffer volume ratio of 1: 8. Lysis was performed using a high pressure homogenizer (AVESTIN, 4 cycles at 14000 psi). After lysis, urea was added at final concentration of 5M, followed by agitation for 1 hour at room temperature. The pH was reduced from 6 to 5 using 200 mM acetate buffer (pH 4)+ 5 M urea. The mixture was centrifuged at 16800g for 60 minutes at 2-8°C. The supernatant was collected and filtered by SARTOBRAN P (0.45-0.22μm SARTORIUS).

Protein in the filtered supernatant was stable for at least 30 days at –20°C and for at least 15 days at 2-8°C.

Protein was further purified on a cationic exchange column (SPFF, Amersham Biosciences) with elution using 350mM NaCl + 50 mM acetate + 5 M urea pH 5.00. The majority of impurities were present in the flow-thru. A pre-elution washing using a lower NaCl concentration (180 mM) advantageously eliminated two contaminating *E.coli* proteins .

The eluted material was adjusted to pH 8 (using 200 mM TRIS/HCl + 5 M urea pH 9) and further purified on a Q Sepharose HP column (Amersham) with elution using 150 mM NaCl + 20 mM TRIS/HCl pH 8.00 in 5 M urea. Again, a pre-elution washing with reduced salt (90 mM) was useful for eliminating impurities.

The filtered eluted material from Q HP column was diluted 1:2 using PBS pH 7.00 (150 mM NaCl + 10 mM potassium phosphate, pH 7.00) and then diafiltered against 10 volumes of PBS pH 7.00 by tangential ultrafiltration. At the end of diafiltration the material was concentrated 1.6 times to about 1.2 mg/ml total proteins. Using a 30,000 Da cut-off membrane (Regenerated Cellulose membrane 50cm^2, Millipore PLCTK 30) it was possible to dialyze the material with a yield of about 90% .

936-ΔG741 hybrid protein

DNA encoding protein 936 from meningococcal serogroup B strain 2996 and protein 741 from meningococcal serogroup B strain MC58 were digested and ligated, together with a short linker

sequence, to give a plasmid encoding amino acid sequence SEQ ID 8. The plasmid was transfected into *E.coli* and bacteria were grown to express the protein. The recombinant protein was not secreted, but remained soluble within the bacteria.

After adequate growth, bacteria were centrifuged to give a humid paste and treated as follows:

- Homogenisation by high pressure system in presence of 20mM sodium phosphate pH 7.00.
- Centrifugation and clarification by orthogonal filtration.
- Cationic column chromatography (SP Sepharose Fast Flow), with elution by 150mM NaCl in 20mM sodium phosphate pH 7.00.
- Anionic column chromatography (Q Sepharose XL) with flow-through harvesting.
- Hydrophobic column chromatography (Phenyl Sepharose 6 Fast Flow High Sub) with elution by 20mM sodium phosphate, pH 7.00.
- Diafiltration against PBS pH 7.4 with a 10Kd cut-off.
- Final sterile filtration and storing at –20°C

Protein in the final material was stable for at least 3 months both at –20°C and at 2-8°C.

961$^{(NL)(C)}$ protein

DNA encoding protein 936 from meningococcal serogroup B strain 2996 was digested to remove the sequence encoding its C-terminus, to give a plasmid encoding amino acid sequence SEQ ID 1. The plasmid was transfected into *E.coli* and bacteria were grown to express the protein. The recombinant protein was secreted into the culture meduim, and the leader peptide was absent in the secreted protein (SEQ ID 2). The supernatant was treated as follows:

- Concentration 7X and diafiltration against buffer 20mM TRIS/HCl pH7.6 by cross flow UF (Cut off 30Kd).
- Anionic column chromatography (Q Sepharose XL), with elution by 400mM NaCl in 20mM TRIS/HCl pH 7.6.
- Hydrophobic column chromatography step (Phenyl Sepharose 6 Fast Flow High Sub), with elution by 50mM NaCl in TRIS/HCl pH 7.6.
- Hydroxylapatite ceramic column chromatography (HA Macro. Prep) with elution by 200mM sodium phosphate pH 7.4.
- Diafiltration (cut off 30Kd) against PBS pH 7.4
- Final sterile filtration and storing at –20°C

Protein in the final material was stable for at least 6 months both at –20°C and at 2-8°C.

Antigenic combinations

Mice were immunised with a composition comprising the three proteins and an aluminium hydroxide adjuvant. For comparison purposes, the three proteins were also tested singly. Ten mice were used per group. The mixture was able to induce high bactericidal titres against various strains:

	Meningococcal strain (Serogroup)							
	2996 (B)	MC58 (B)	NGH38	394/98 (B)	H44/76 (B)	F6124 (A)	BZ133 (C)	C11 (C)
(1)	32000	16000	130000	16000	32000	8000	16000	8000
(2)	256	131000	128	16000	32000	8000	16000	<4
(3)	32000	8000	—	—	—	8000	—	32000
Mix	**32000**	**32000**	**65000**	**16000**	**260000**	**65000**	**>65000**	**8000**

'—' indicates that this strain contains no NadA gene

Looking at individual mice, the triple mixture induced high and consistent bactericidal titres against the three serogroup B strains from which the individual antigens are derived:

#	1	2	3	4	5	6	7	8	9	10
2996	32768	16384	65536	32768	32768	65536	65536	32768	65536	8192
MC58	65536	32768	65536	65536	65536	8192	65536	32768	32768	65536
394/98	65536	4096	16384	4096	8192	4096	32768	16384	8192	16384

Combination and comparison with OMVs

In further experiments, the adjuvanted antigens (20μg of each antigen per dose) were administered in combination with 10μg OMVs prepared either from strain H44/76 (Norway) or strain 394/98 (New Zealand). Positive controls were the anti-capsular SEAM-3 mAb for serogroup B or CRM197-conjugated capsular saccharides for other strains. Results (bactericidal titres) are shown in Table 1. The mixture almost always gives better titres than simple OMVs and, furthermore, the addition of the mixture to OMVs almost always significantly enhances the efficacy of the OMVs. Moreover, in many cases the antigen mixture matches or exceeds the response seen with the positive control.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

TABLE 1

	2996	NGH38	M4215	MC58	44/76	CU385	N44/89	394/98	M01-240149	NM092	NM008	BZ198	961-5945	G2136	5/99		F6124	BZ133	LPN17592	240539
Typing:	B:2b:P1.5a,2a	B:NT: P1.3	B:15:P1.7,16	B:15:P1.7,16b	B:15:P1.7,16	B:4:P1.15	B:4,7:P1.19,15	B:4:P1.4	B:4:P1.7,4	B:4:P1.4	B:4:P1.4	B:NT	B:2b:P1.21,16	B:	B:2b:P1.5,2		A	C:NT:	W135	P1.5
ET:	other	other	n.d.	ET5	ET5	ET5	ET5	lin.3	lin.3	lin 3	lin 3	lin 3	A4	A4	A4		sIII	sI		
Positive control	32768	32768	32768	16384	16384	>16384	8192	16384	8192	32768	8192	16384	8192	32768	8192		1024		1024	4096
Antigen mixture	4096	4096	65536	32768	65536	>65536	>4096	8192	2048	>4096	4096	4096	2048	2048	>4096		8192	16384	4096	>8192
Antigens + H44/76 OMVs	16384	8192	>65536	32768	524288	>65536	>4096	16384	8192	>4096	>4096	>4096	>8192	2048	>4096		32768	32768	16384	>8192
Antigens + 394/98 OMV	8192	8192	>65536	32768	>65536	>65536	>4096	65536	>8192	>4096	>4096	>4096	2048	8192	>4096		65536	65536	65536	>8192
OMVs (Norway)	<4	1024	8192	2048	262144	256	<8	4096	<4	<8	<8	<4	>8192	<8	<8		1024	<4	<8	>4096
OMVs (NZ)	512	<4	128	2048	<4	<8	<8	32768	>8192	4096	1024	4096	<16	n.d.	<8		4096	1024	<8	>4096

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CLAIMS

1. A composition comprising meningococcal antigens: (1) a 'NadA' protein; (2) a '741' protein; (3) a '936' protein; (4) a '953' protein; and (5) a '287' protein.

2. The composition of claim 1, wherein the NadA protein has 85% or more identity to SEQ ID 2.

3. The composition of claim 2, wherein the NadA protein comprises SEQ ID 2.

4. The composition of any preceding claim, wherein the 741 protein has 85% or more identity to SEQ ID 3.

5. The composition of claim 4, wherein the 741 protein comprises SEQ ID 3.

6. The composition of any preceding claim, wherein the 936 protein has 85% or more identity to SEQ ID 4.

7. The composition of claim 6, wherein the 936 protein comprises SEQ ID 4.

8. The composition of any preceding claim, wherein the 953 protein has 85% or more identity to SEQ ID 5.

9. The composition of claim 8, wherein the 953 protein comprises SEQ ID 5.

10. The composition of any preceding claim, wherein the 287 protein has 85% or more identity to SEQ ID 6.

11. The composition of claim 10, wherein the 287 protein comprises SEQ ID 6.

12. The composition of any preceding claim, wherein at least two of the antigens (1) to (5) are expressed as a single polypeptide chain.

13. The composition of claim 12, wherein the composition comprises a polypeptide which comprises a pair of antigens within a single polypeptide chain selected from the group consisting of: NadA & 741; NadA & 936; NadA & 953; NadA & 287; 741 & 936; 741 & 953; 741 & 287; 936 & 953; 936 & 287; 953 & 287.

14. The composition of claim 12 or claim 13, wherein the composition comprises a polypeptide of formula NH_2-A-[-X-L-]$_n$-B-COOH, wherein: X is an amino acid sequence of one of the five antigens (1) to (5); L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and n is 2, 3, 4 or 5.

15. The composition of claim 14, wherein n is 2, X_1 is a 936 protein and X_2 is a 741 protein.

16. The composition of claim 14, wherein n is 2, X_1 is a 287 protein and X_2 is a 953 protein.

17. The composition of any preceding claim, comprising a protein comprising SEQ ID 7.

18. The composition of any preceding claim, comprising a protein comprising SEQ ID 8.

19. The composition of any preceding claim, for use as a medicament.

20. The use of a composition of any preceding claim in the manufacture of a medicament for the prevention and/or treatment of a disease caused by a *Neisseria*.

21. A method for raising an antibody response in a mammal, comprising the step of administering an effective amount of a composition according to any one of claims 1 to 18.

22. A polypeptide having an amino acid sequence selected from the group consisting of SEQ IDs 1 to 8.

23. A process for purifying soluble NadA from a culture medium, comprising the steps of: concentration and diafiltration against a buffer by ultrafiltration; anionic column chromatography; hydrophobic column chromatography; hydroxylapatite ceramic column chromatography; diafiltration against a buffer; and filter sterilisation.

24. A process for purifying a 936-ΔG741 hybrid protein from a bacterium, comprising the steps of: homogenisation; centrifugation; cationic column chromatography; anionic column chromatography; hybrophobic column chromatography; diafiltration against a buffer; and filter sterilisation.

SEQUENCE LISTING

SEQ ID 1 – 961 from strain 2996, with C-terminus deletion

MKHFPSKVLTTAILATFCSGALAATNDDDVKKAATVAIAAAYNNGQEINGFKAGETIYDIDEDGTITKKDATAADVEADDFKGLGLKK
VVTNLTKTVNENKQNVDAKVKAAESEIEKLTTKLADTDAALADTDAALDATTNALNKLGENITTFAEETKTNIVKIDEKLEAVADTVD
KHAEAFNDIADSLDETNTKADEAVKTANEAKQTAEETKQNVDAKVKAAETAAGKAEAAAGTANTAADKAEAVAAKVTDIKADIATNKD
NIAKKANSADVYTREESDSKFVRIDGLNATTEKLDTRLASAEKSIADHDTRLNGLDKTVSDLRKETRQGLAEQAALSGLFQPYNVG

SEQ ID 2 – 961 from strain 2996, with C-terminus deletion and leader peptide processed

ATNDDDVKKAATVAIAAAYNNGQEINGFKAGETIYDIDEDGTITKKDATAADVEADDFKGLGLKKVVTNLTKTVNENKQNVDAKVKAA
ESEIEKLTTKLADTDAALADTDAALDATTNALNKLGENITTFAEETKTNIVKIDEKLEAVADTVDKHAEAFNDIADSLDETNTKADEA
VKTANEAKQTAEETKQNVDAKVKAAETAAGKAEAAAGTANTAADKAEAVAAKVTDIKADIATNKDNIAKKANSADVYTREESDSKFVR
IDGLNATTEKLDTRLASAEKSIADHDTRLNGLDKTVSDLRKETRQGLAEQAALSGLFQPYNVG

SEQ ID 3 – ΔG741 from MC58 strain

VAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGE
FQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKIEH
LKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNGIRHIGLAAKQ

SEQ ID 4 – 936 from MC58 strain with leader peptide processed

VSAVIGSAAVGAKSAVDRRTTGAQTDDNVMALRIETTARSYLRQNNQTKGYTPQISVVGYNRHLLLLGQVATEGEKQFVGQIARSEQA
AEGVYNYITVASLPRTAGDIAGDTWNTSKVRATLLGISPATQARVKIVTYGNVTYVMGILTPEEQAQITQKVSTTVGVQKVITLYQNY
VQR

SEQ ID 5 – 953 from MC58 strain with leader peptide processed

ATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPIANLQSGSQHFTDHLKSADIFDAAQYPDIRFVSTKFN
FNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMEKTEVCGGDFSTTIDRTKWGMDYLVNVGMTKSVRIDIQIEAAKQ

SEQ ID 6 – ΔG287 from MC58 strain

SPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNDMPQNAAGTDSS
TPNHTPDPNMLAGNMENQATDAGESSQPANQPDMANAADGMQGDDPSAGGQNAGNTAAQGANQAGNNQAAGSSDPIPASNPAPANGGS
NFGRVDLANGVLIDGPSQNITLTHCKGDSCSGNNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDKFVGLVADSVQMKGINQYIIFYK
PKPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEPAKGEMLAG
AAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAG
KYSYRPTDAEKGGFGVFAGKKEQD

SEQ ID 7 – 287-953 hybrid

MASPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGGQDMAAVSEENTGNGGAAATDKPKNEDEGAQNDMPQNAADTD
SLTPNHTPASNMPAGNMENQAPDAGESEQPANQPDMANTADGMQGDDPSAGGENAGNTAAQGTNQAENNQTAGSQNPASSTNPSATNS
GGDFGRTNVGNSVVIDGPSQNITLTHCKGDSCSGNNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDGKNDKFVGLVADSVQMKGINQ
YIIFYKPKPTSFARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEPSK
GEMLAGTAVYNGEVLHFHTENGRPSPSRGRFAAKVDFGSKSVDGIIDSGDGLHMGTQKFKAAIDGNGFKGTWTENGGGDVSGKFYGPA
GEEVAGKYSYRPTDAEKGGFGVFAGKKEQDGSGGGGATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPV
ANLQSGSQHFTDHLKSADIFDAAQYPDIRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDR
TKWGVDYLVNVGMTKSVRIDIQIEAAKQ*

SEQ ID 8 – 936-741 hybrid

MVSAVIGSAAVGAKSAVDRRTTGAQTDDNVMALRIETTARSYLRQNNQTKGYTPQISVVGYNRHLLLLGQVATEGEKQFVGQIARSEQ
AAEGVYNYITVASLPRTAGDIAGDTWNTSKVRATLLGISPATQARVKIVTYGNVTYVMGILTPEEQAQITQKVSTTVGVQKVITLYQN
YVQRGSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVD
GQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFA
AKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNGIRHIGLAAKQ*

SEQ ID 9 – linker

GSGGGG